

Title: TARGET-DEPENDENT TRANSCRIPTION USING
 DELETION MUTANTS OF N4 RNA POLYMERASE
 Inventor(s): Elena K. Davydova/Lucia B. Rothman-Denes/Gary A. Dahl/
 Svetlana Y. Gerdes/Jerome J. Jendrisak
 Application No.:
 Docket Number: 310307.00006

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Bacteriophage N4 vRNAP promoters

-11
 A/G
 A G
 G:C
 C:G
 X:X'
 X:X'
 3' C:G A A A/T A C C 5'
 -17

FIG. 1

N4 vRNAP and generation of mini-vRNAP

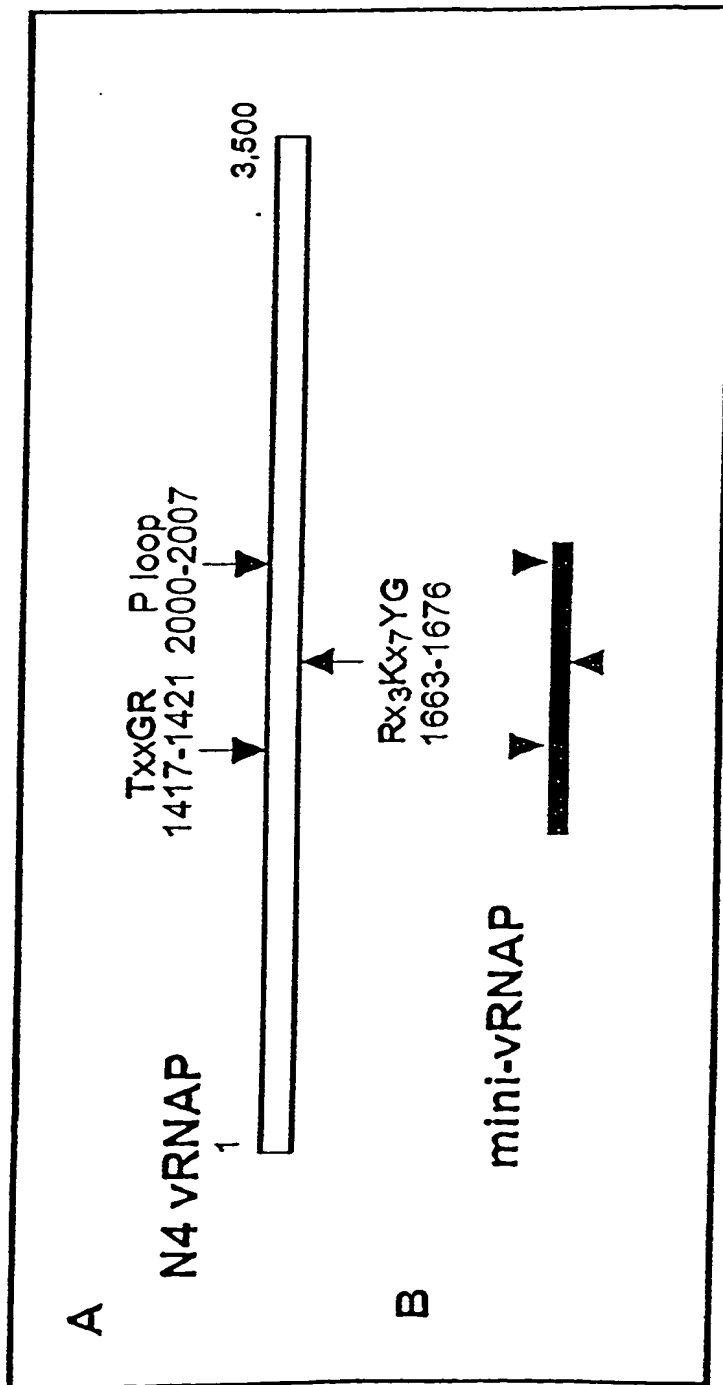


FIG. 2

Identification of the minimal active domain of N4 vRNAP
 by proteolytic cleavage.

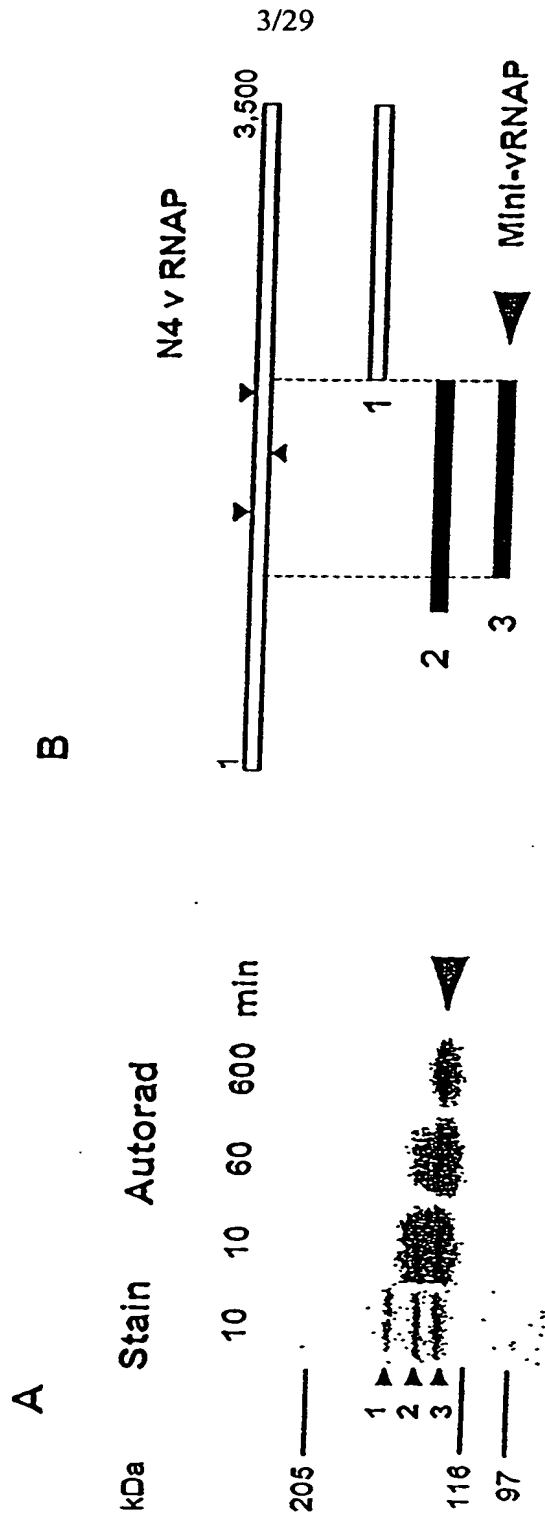
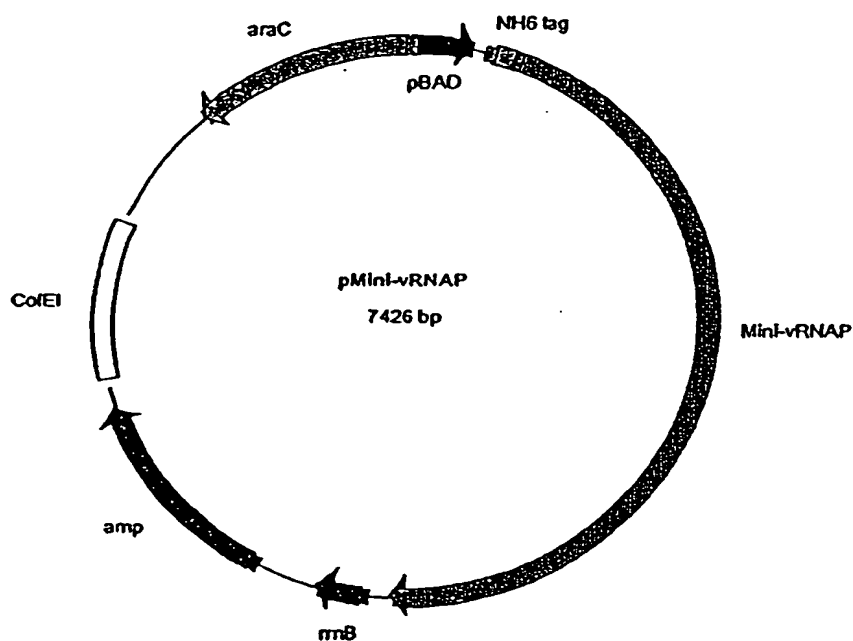


FIG. 3

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Plasmid name: pMini-vRNAP
Plasmid size: 7426 bp
Constructed by: K. M. Kazmierczak
Construction date: 2/2000
Comments: Insert cloned into Invitrogen
pBAD B expression plasmid

FIG 4

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Purification of cloned vRNAP and mini-vRNAP

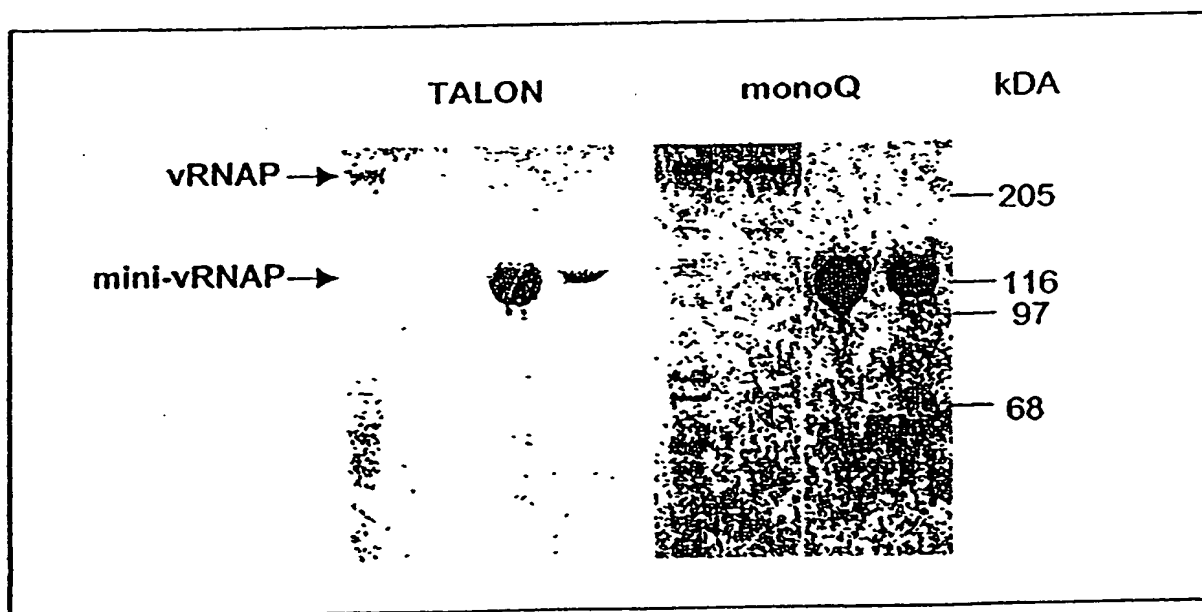


FIG 5

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Activation of N4 vRNAP transcription by *Eco* SSB at different ssDNA concentrations

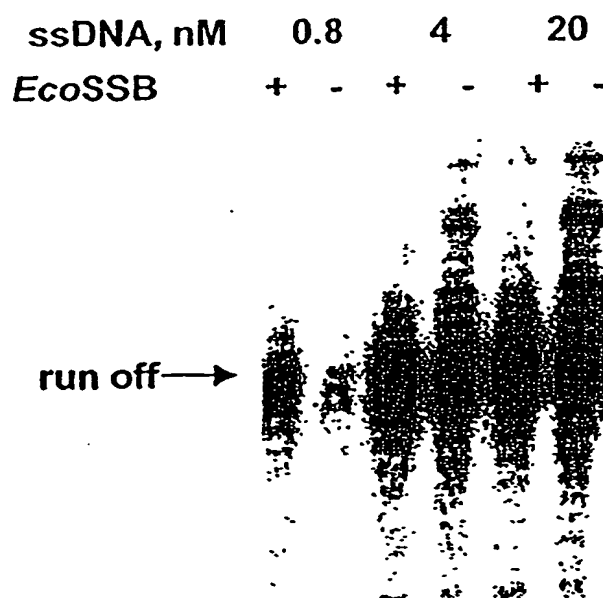


FIG 6

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Effect of Eco SSB on ssDNA template recycling

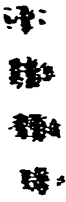
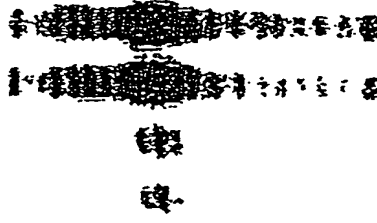
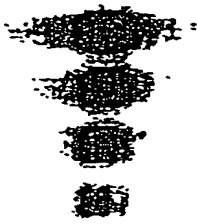
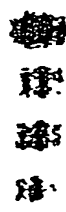
	A	B	C	D
Extra ssDNA	-	+	-	+
Eco SSB	-	-	+	-
time at 37°C	10' 20' 30' 40'	10' 20' 30' 40'	10' 20' 30' 40'	10' 20' 30' 40'
				

FIG. 7

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Effect of *Eco* SSB on the state of template DNA
 and product RNA in vRNAP transcription

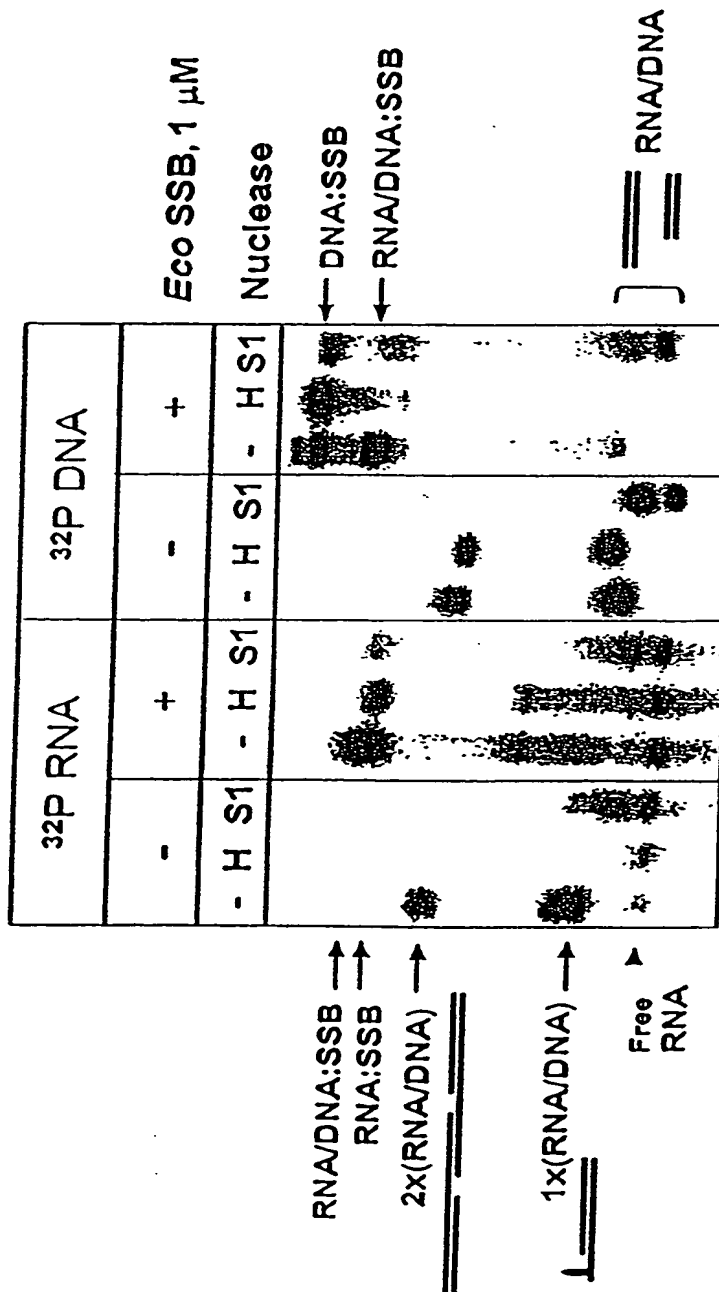


FIG. 8

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Transcription initiation by vRNAP and mini-vRNAP

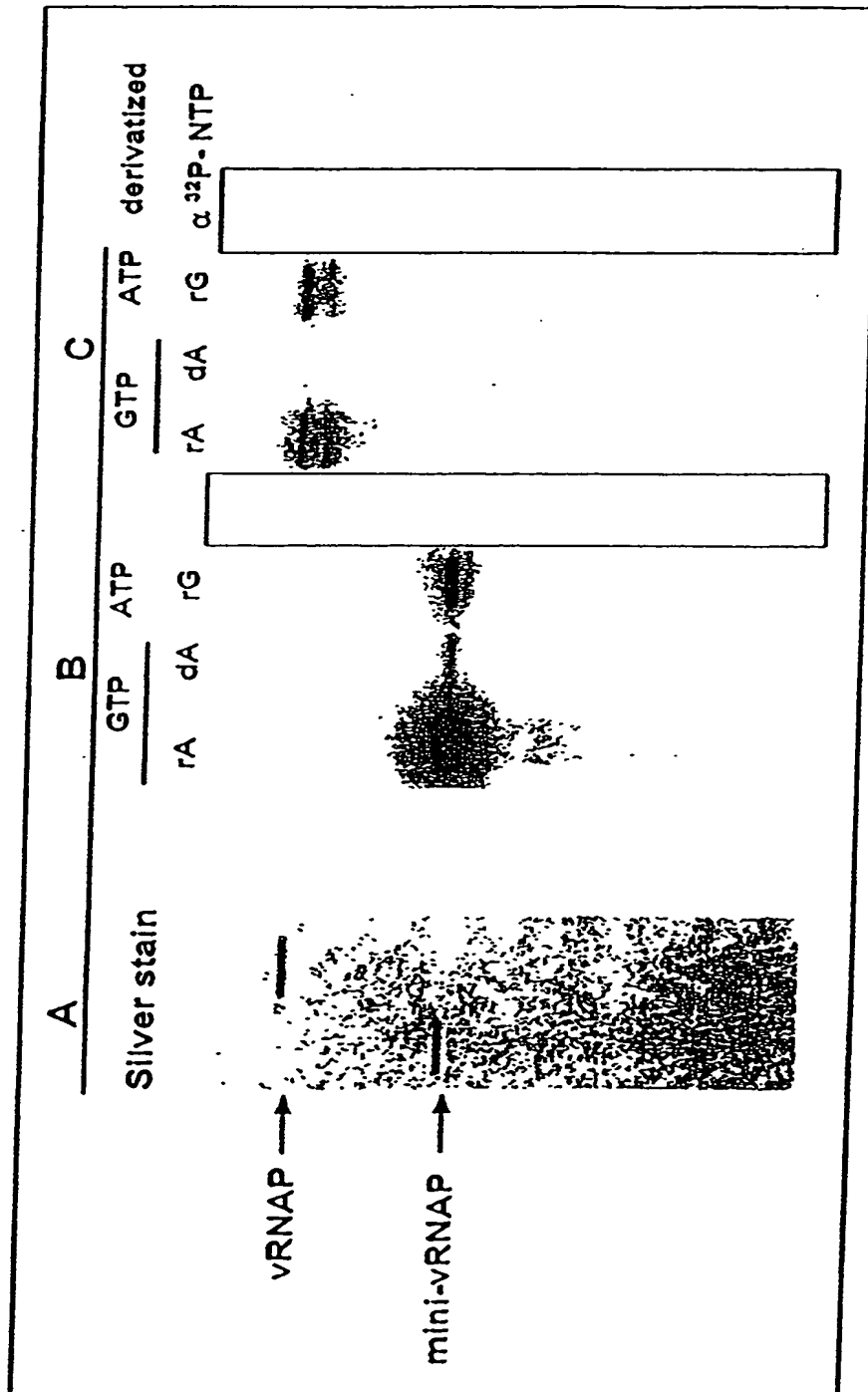


FIG. 9

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Effect of Eco SSB on transcription of vRNAP and mini-RNAP

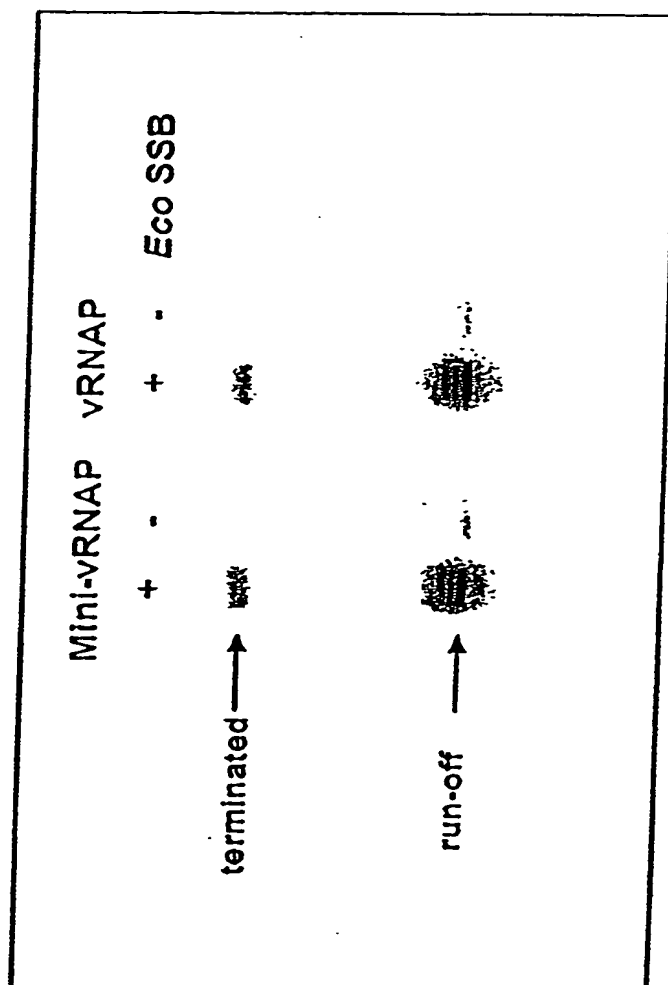


FIG. 10

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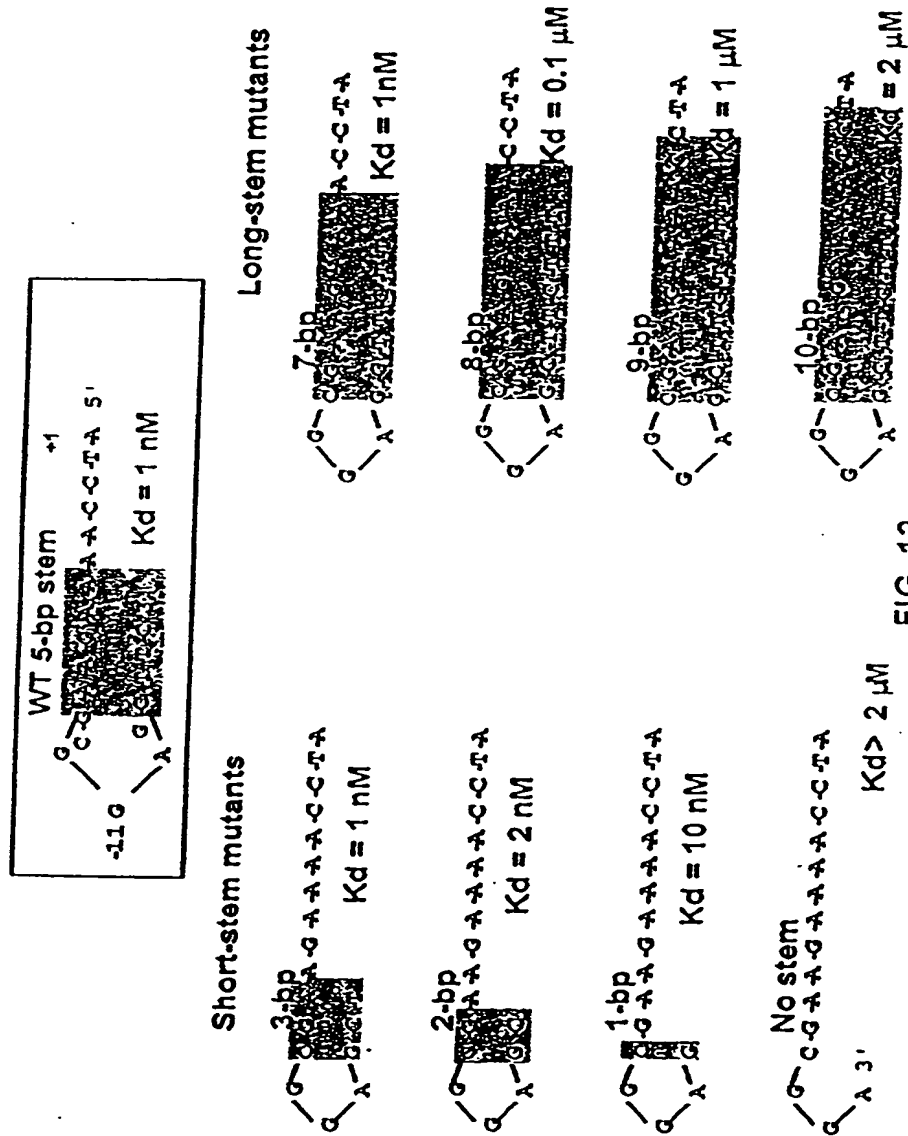
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Binding affinities of stem-length promoter mutants



Identification of the transcription start site by catalytic autolabeling

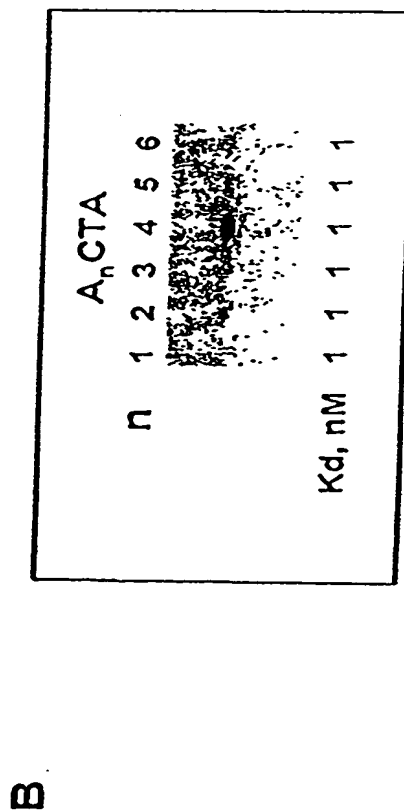
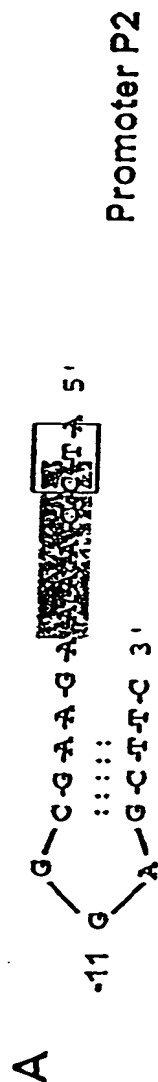
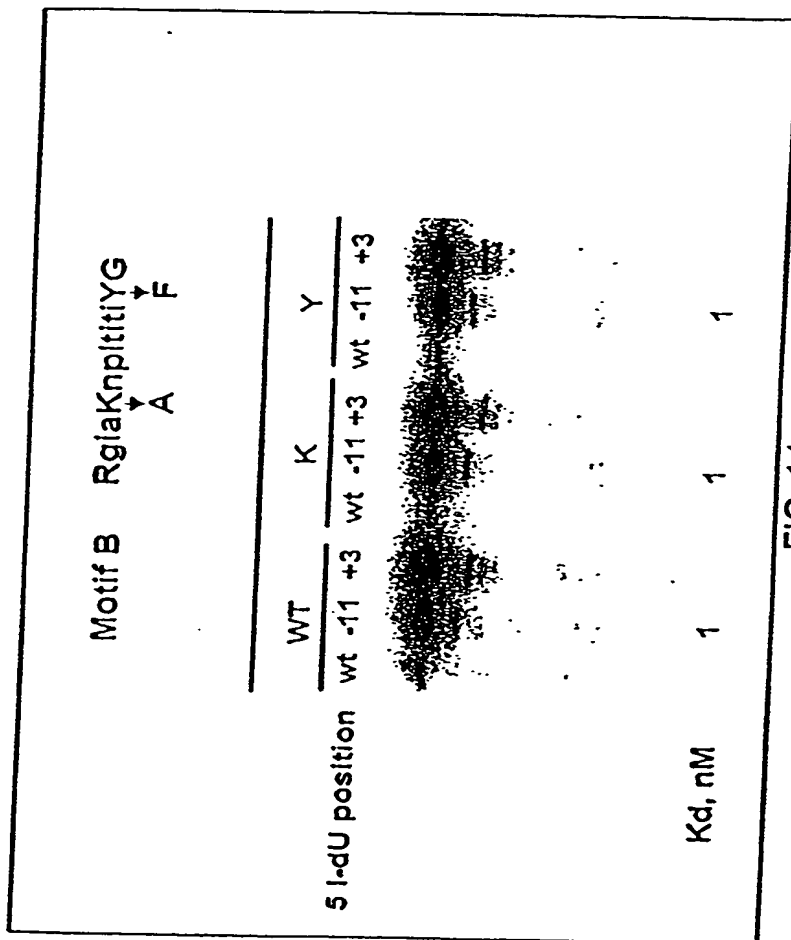


FIG. 13

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UV crosslinking of mutant mini-vRNAPases
 to promoter oligonucleotides



Run-off transcription by mutant mini-vRNAPases

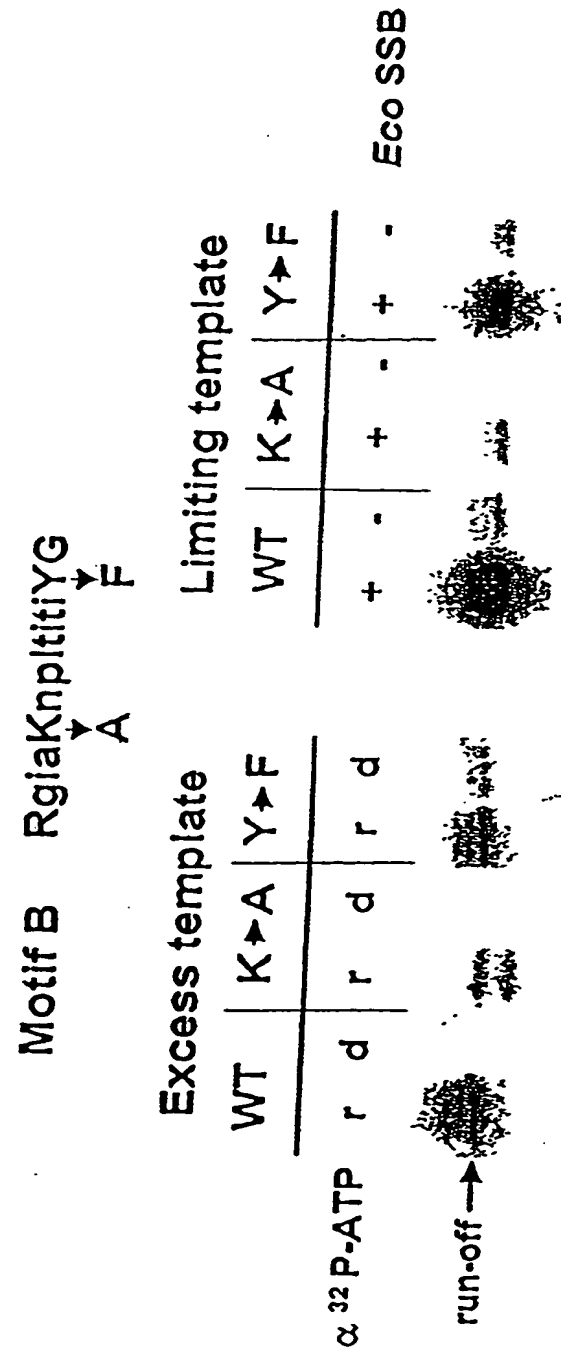


FIG. 15

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Mutant mini-vRNAPases transcription initiation

Motif B RgiaKnplttiY
 A F



FIG. 16

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Detection of *in vivo* activities of N4 vRNAP and mini-vRNAP

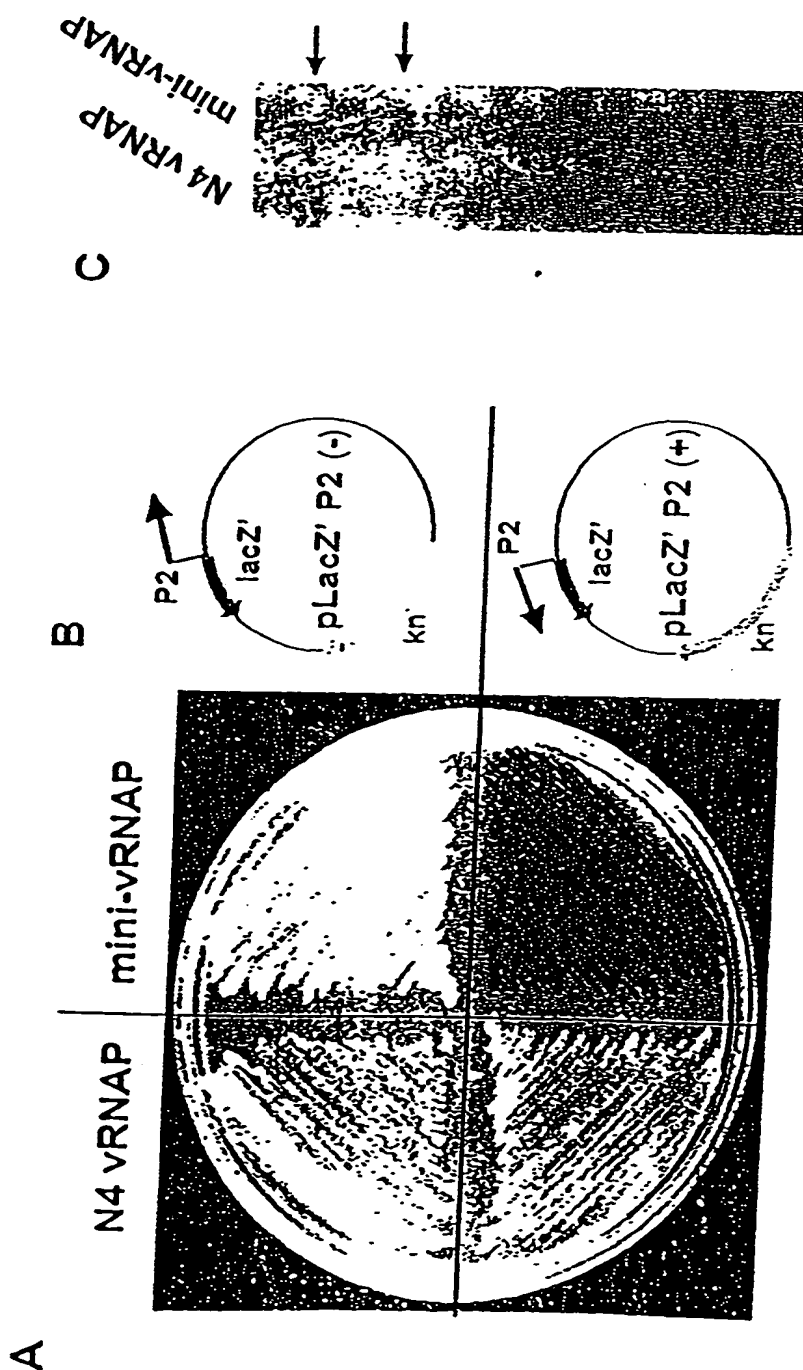
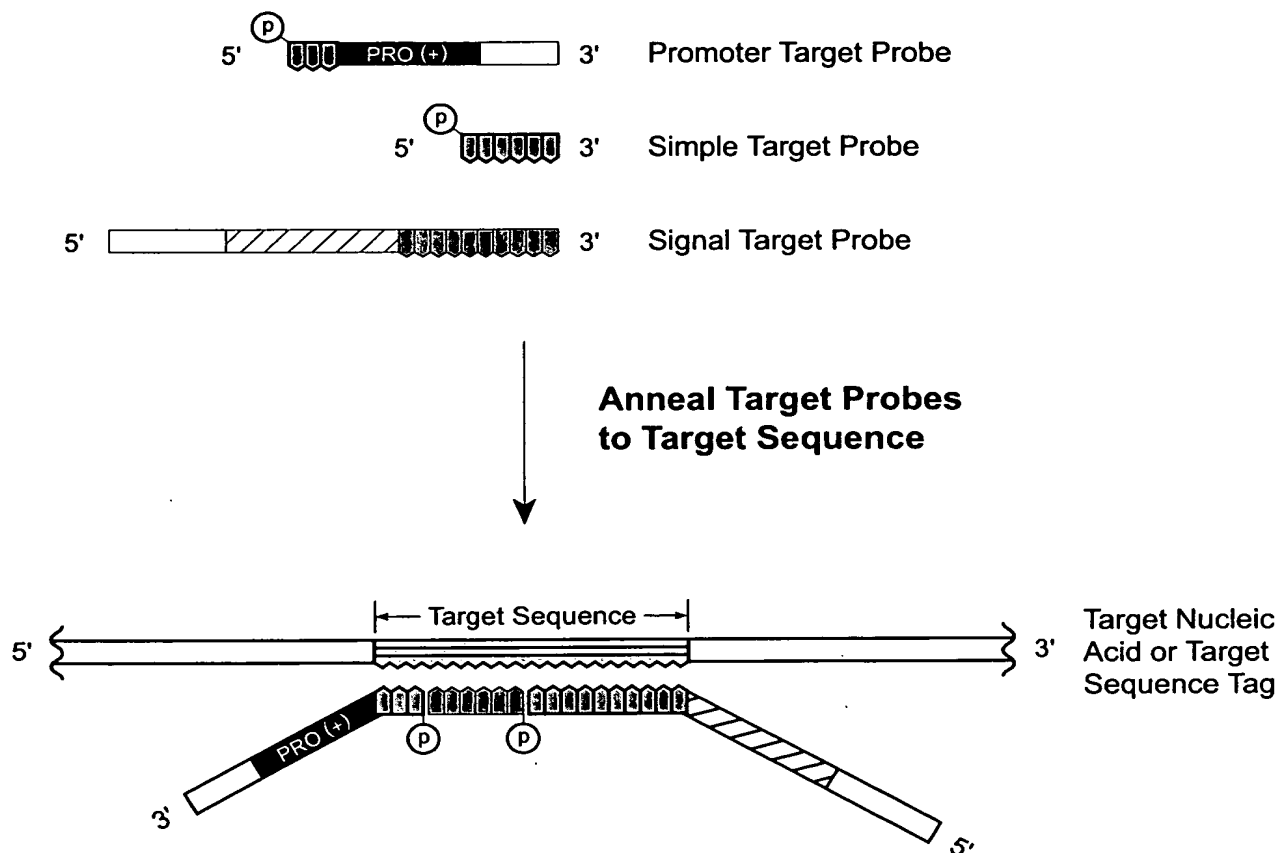


FIG. 17

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Monopartite Target Probes



Portions of a Monopartite Target Probe

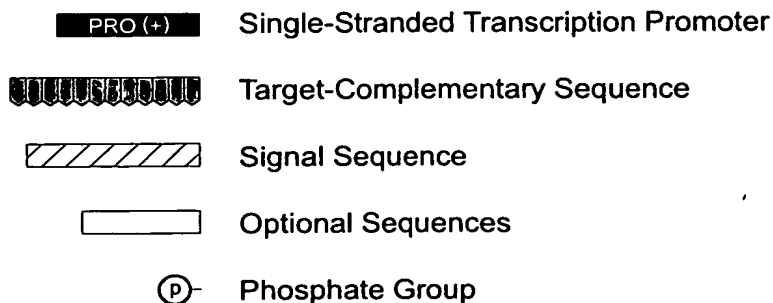
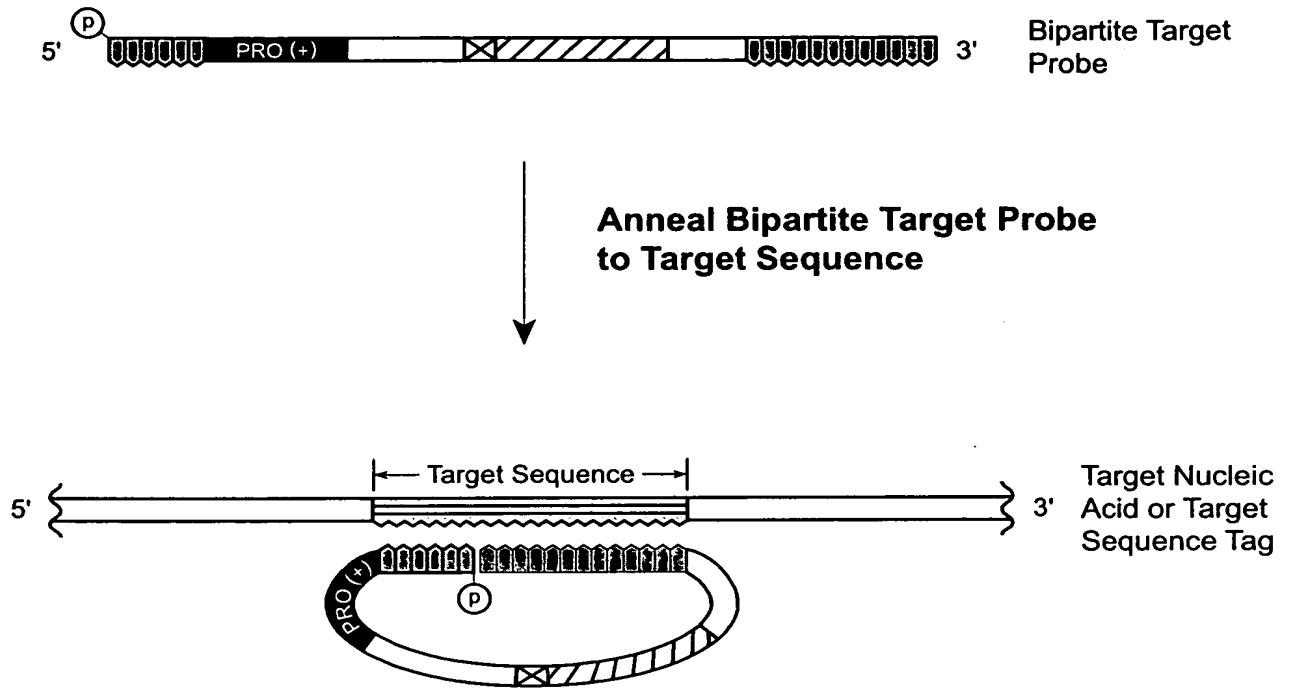


FIG 18

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Portions of a Bipartite Target Probe

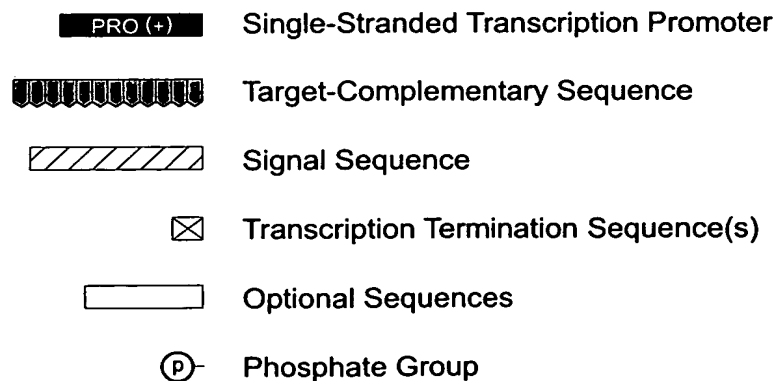


FIG 19

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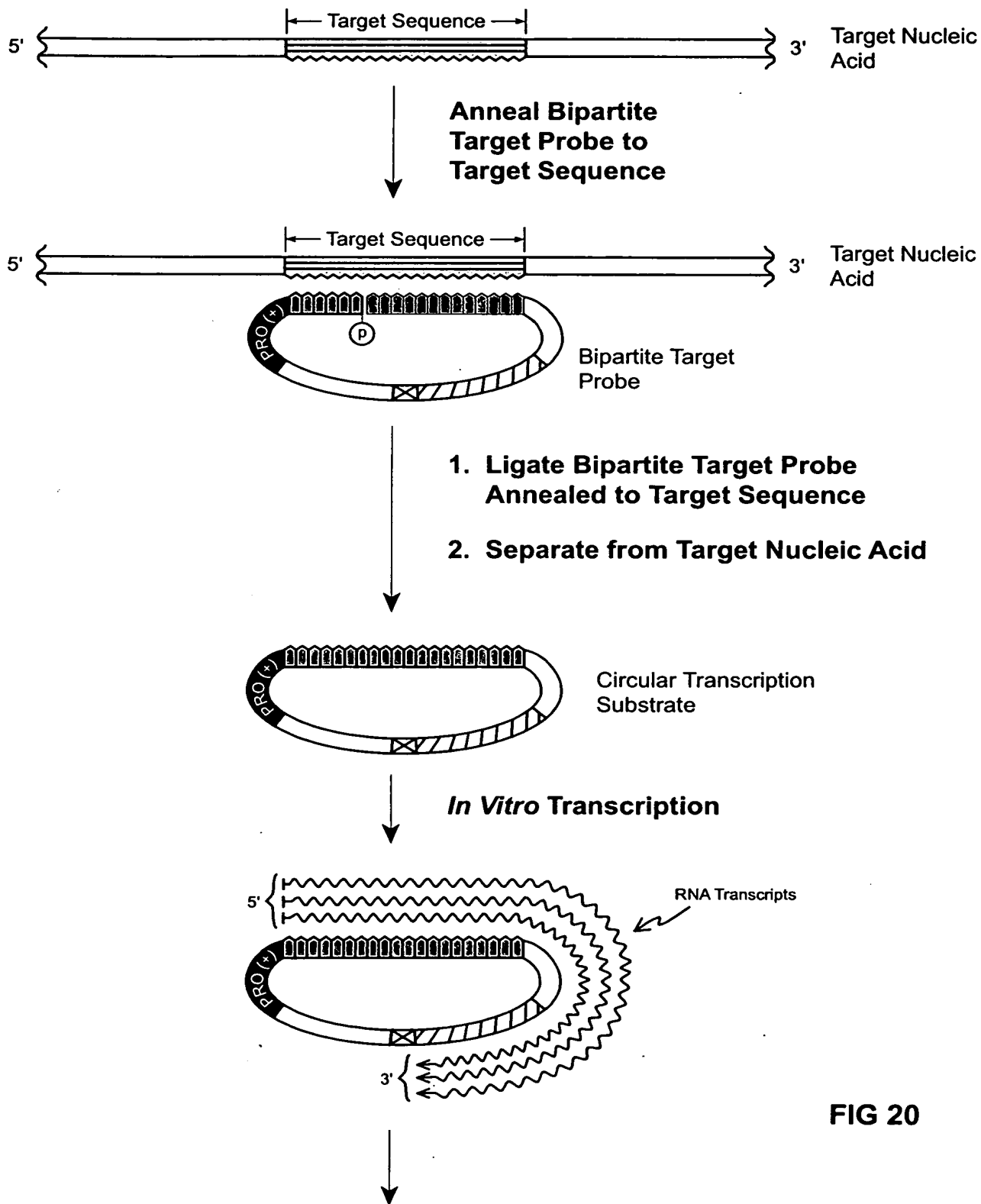


FIG 20

Detect RNA Transcription Products Directly or Indirectly

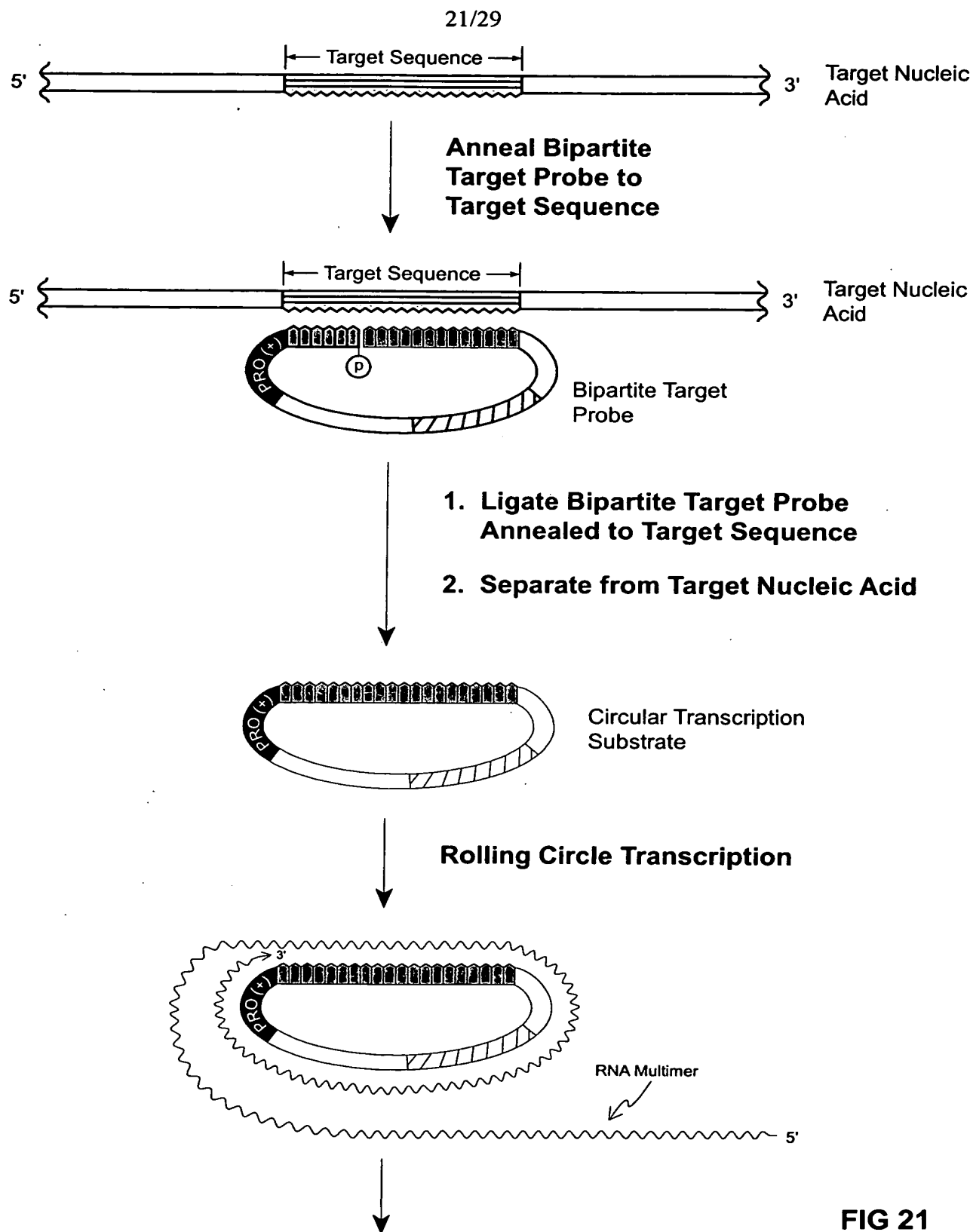


FIG 21

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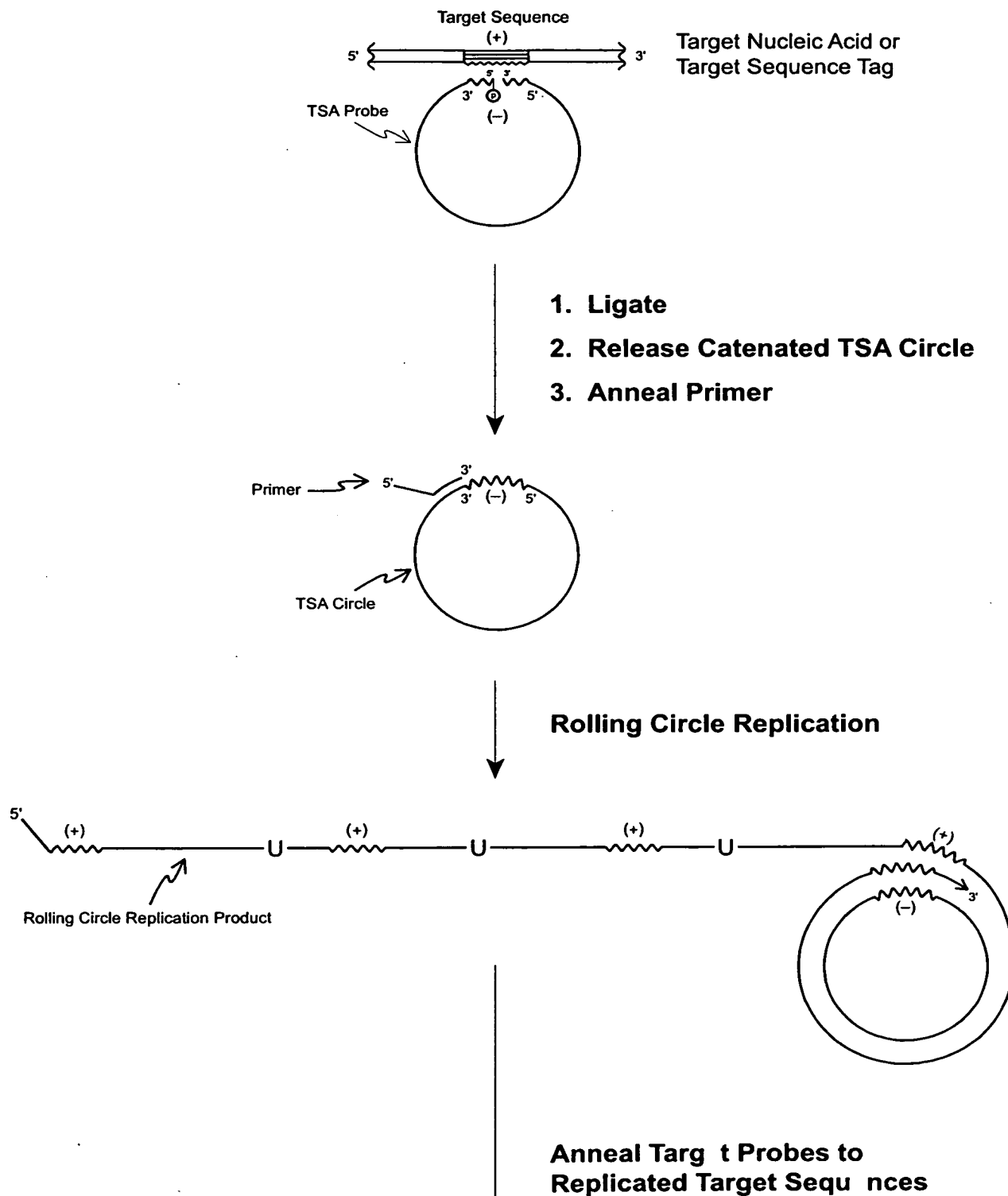


FIG 22A

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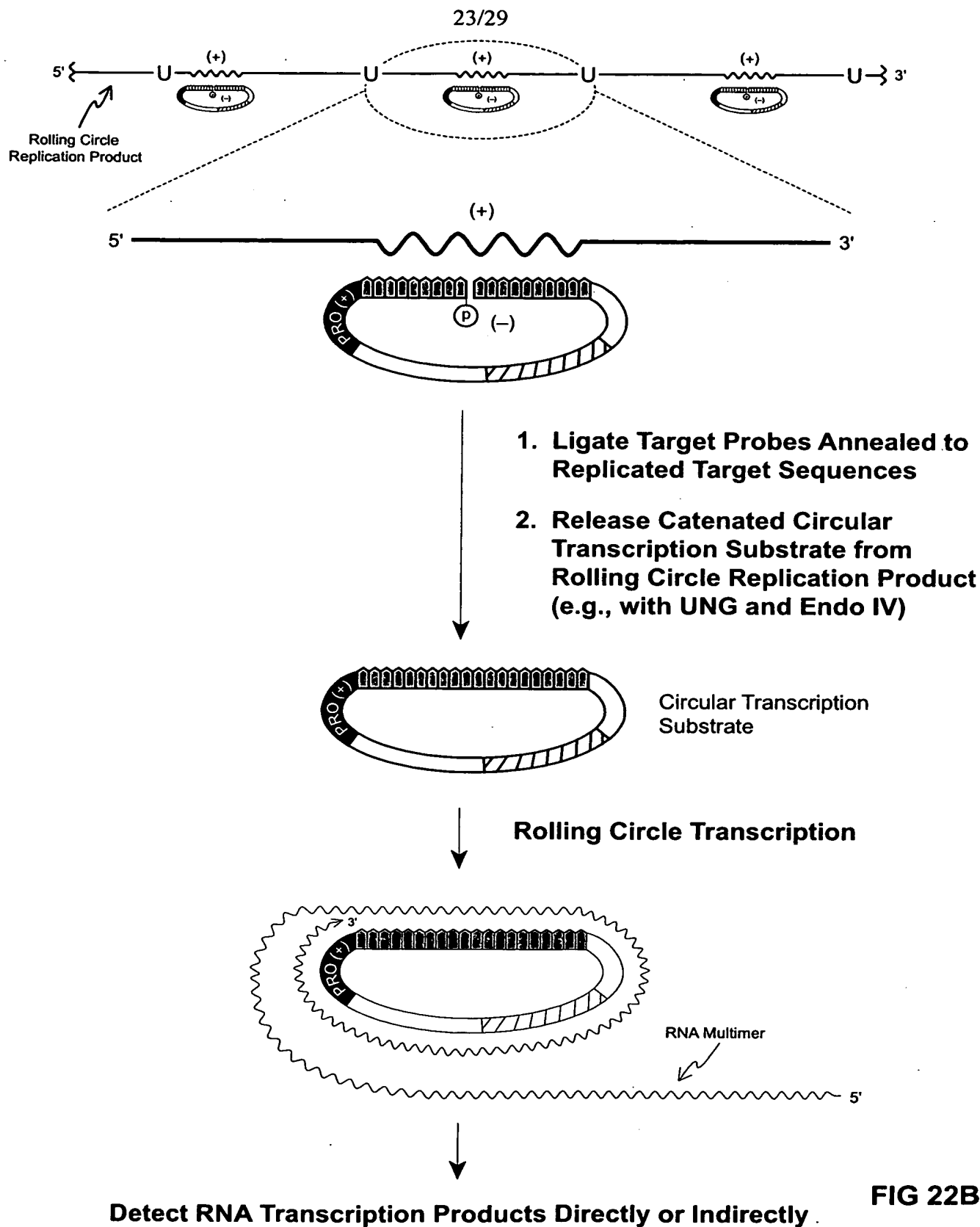
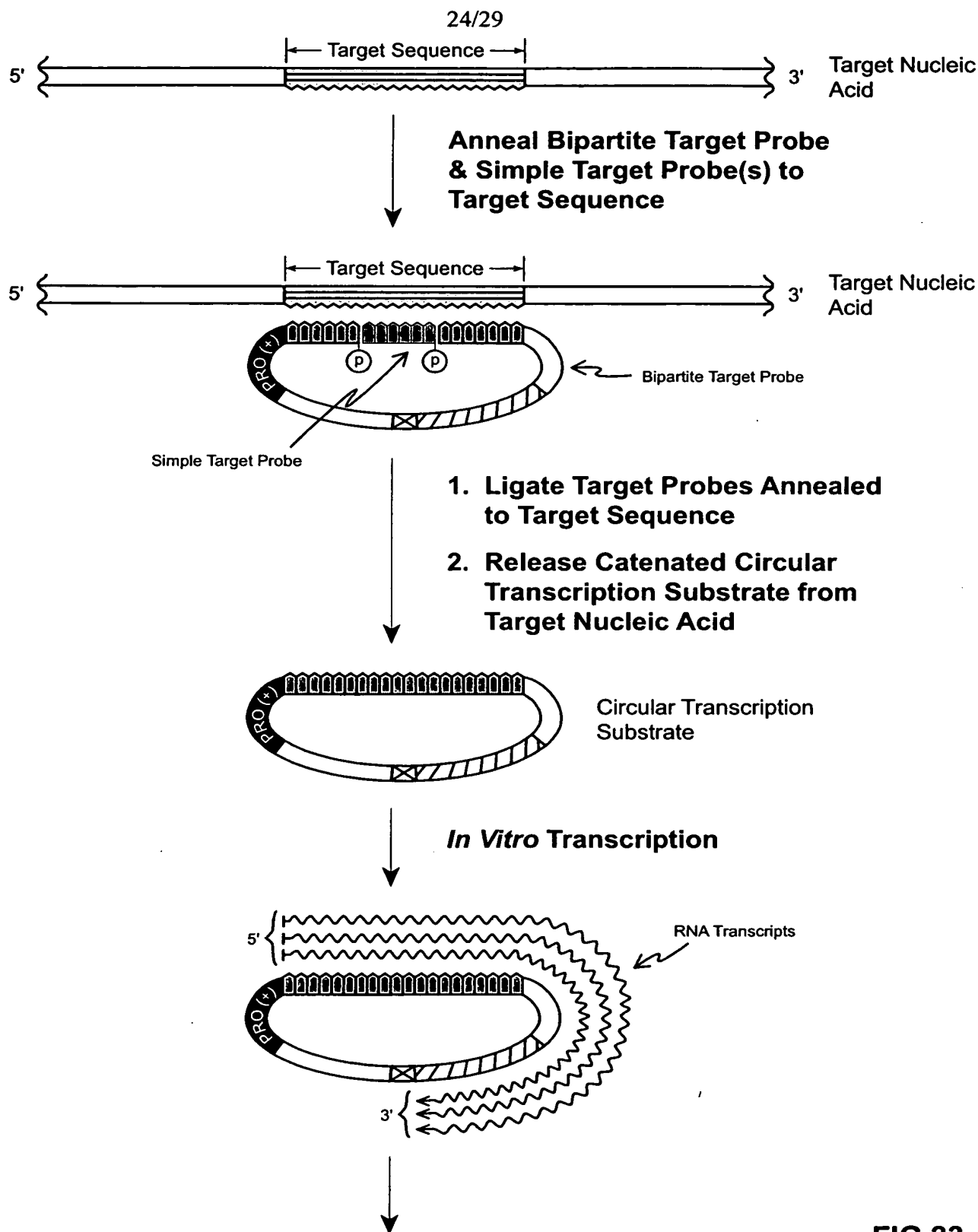


FIG 22B

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Detect RNA Transcription Products Directly or Indirectly

FIG 23

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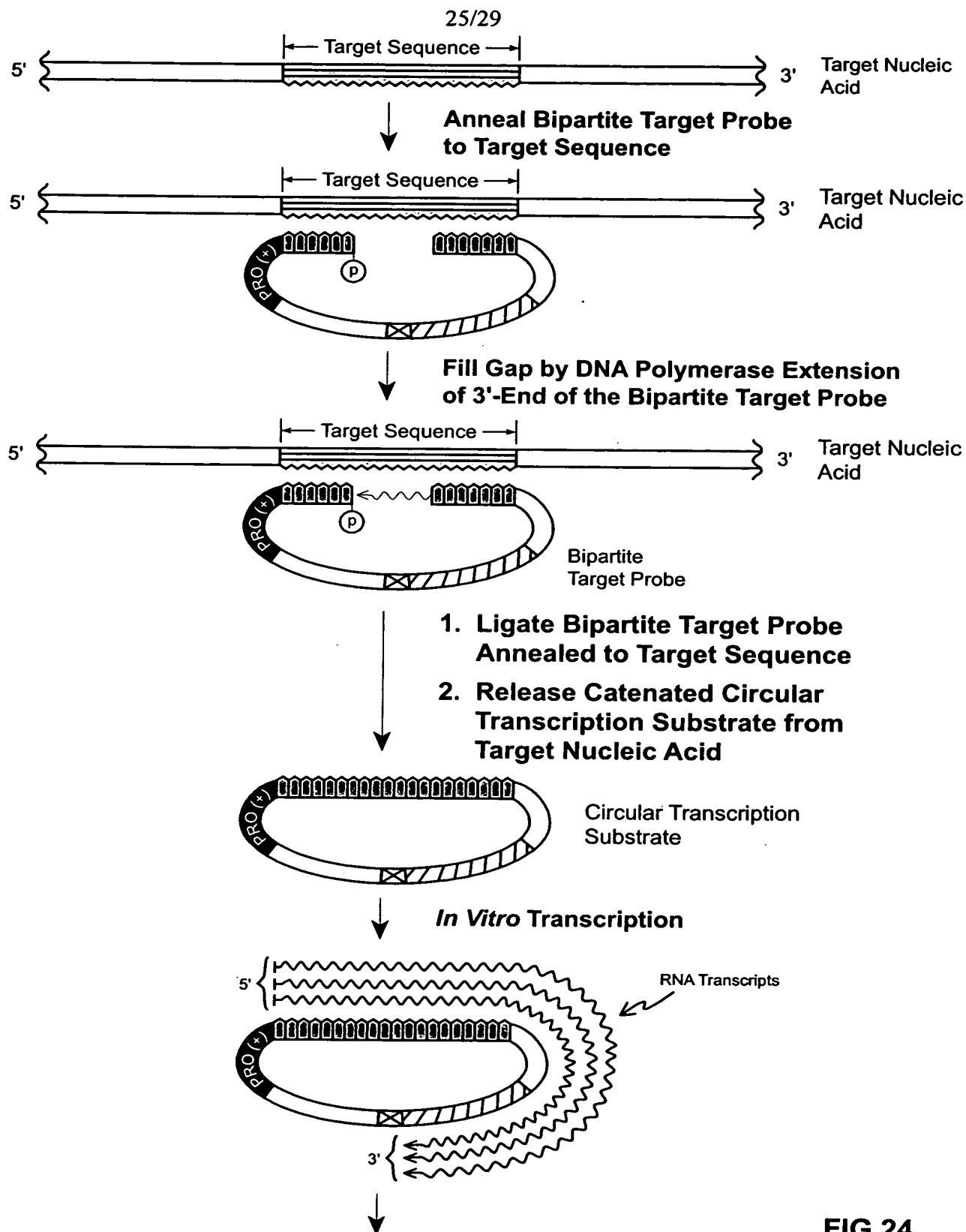


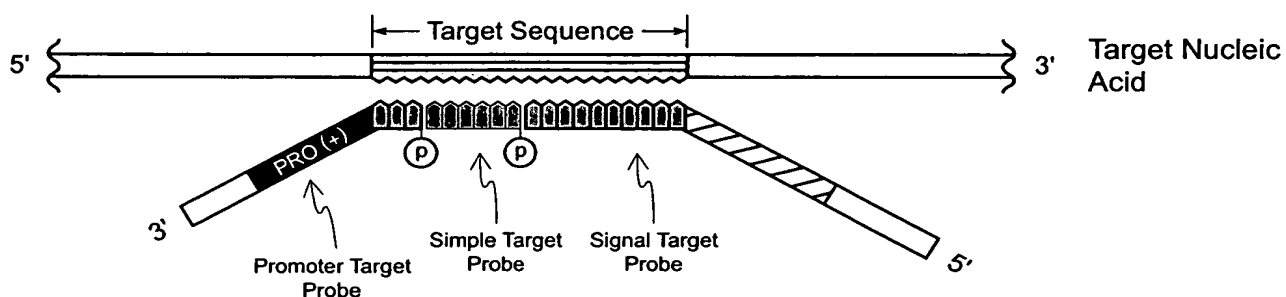
FIG 24

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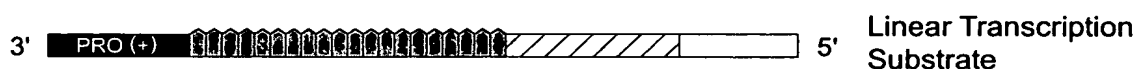
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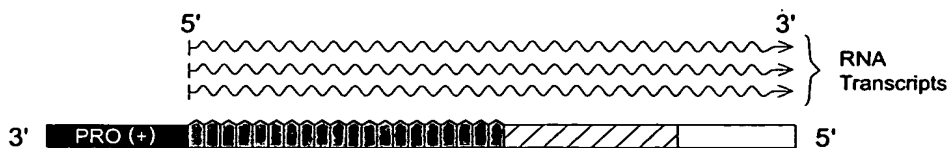
**Anneal Monopartite Target
 Probes to Target Sequence**



**Ligate Target Probes Annealed
 to Target Sequence**



***In Vitro* Transcription**



Detect RNA Transcription Products Directly or Indirectly

FIG 25

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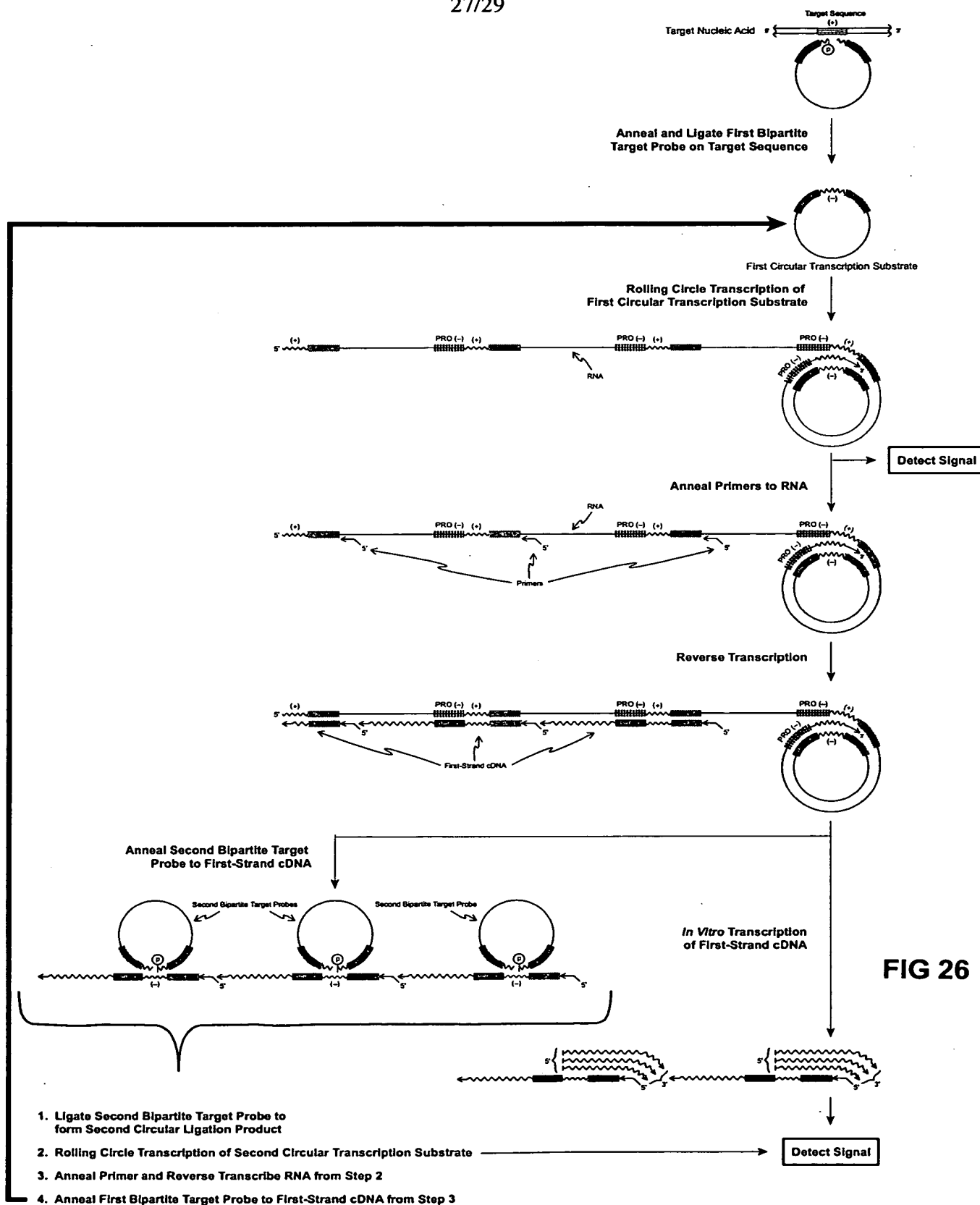


FIG 26

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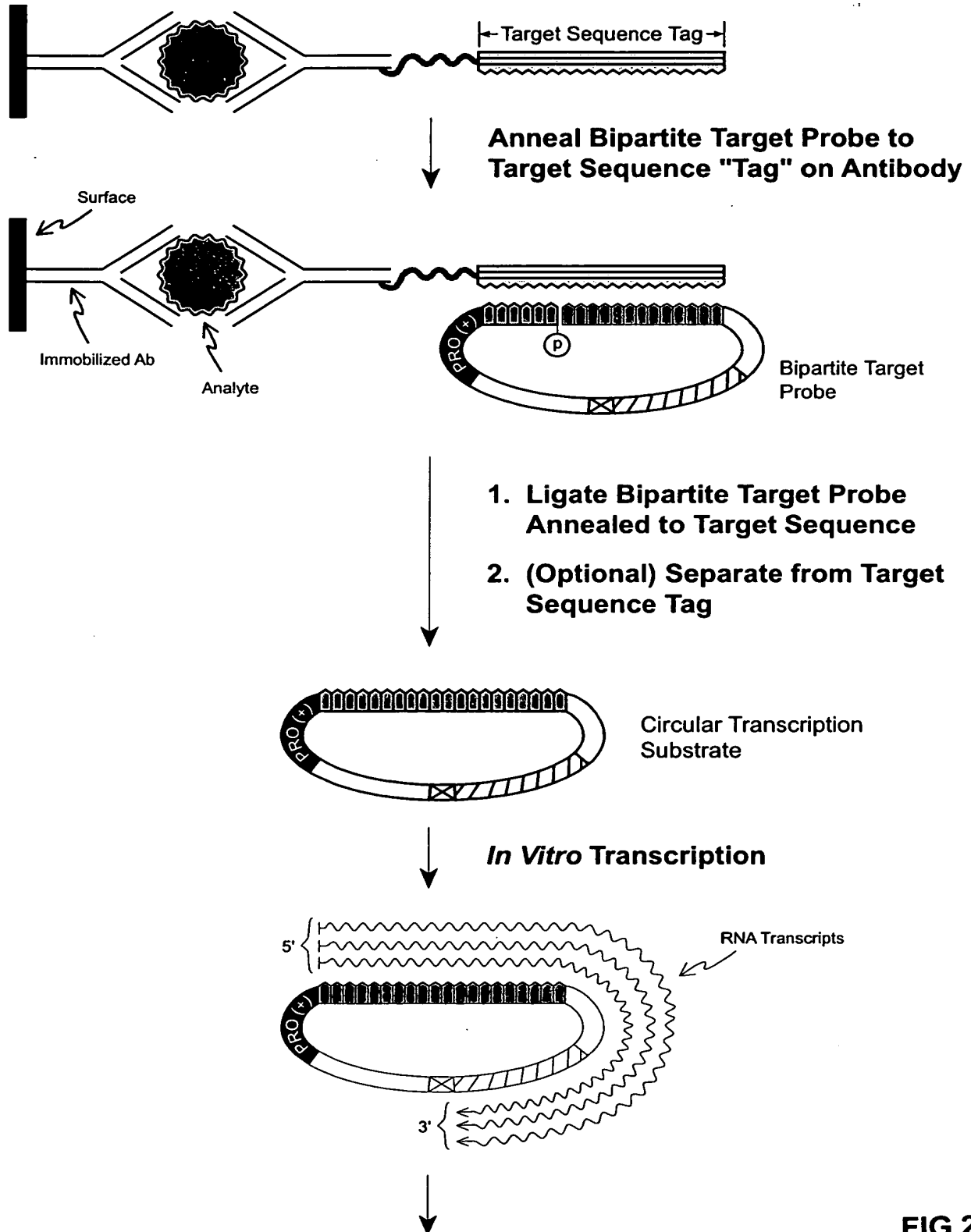


FIG 27

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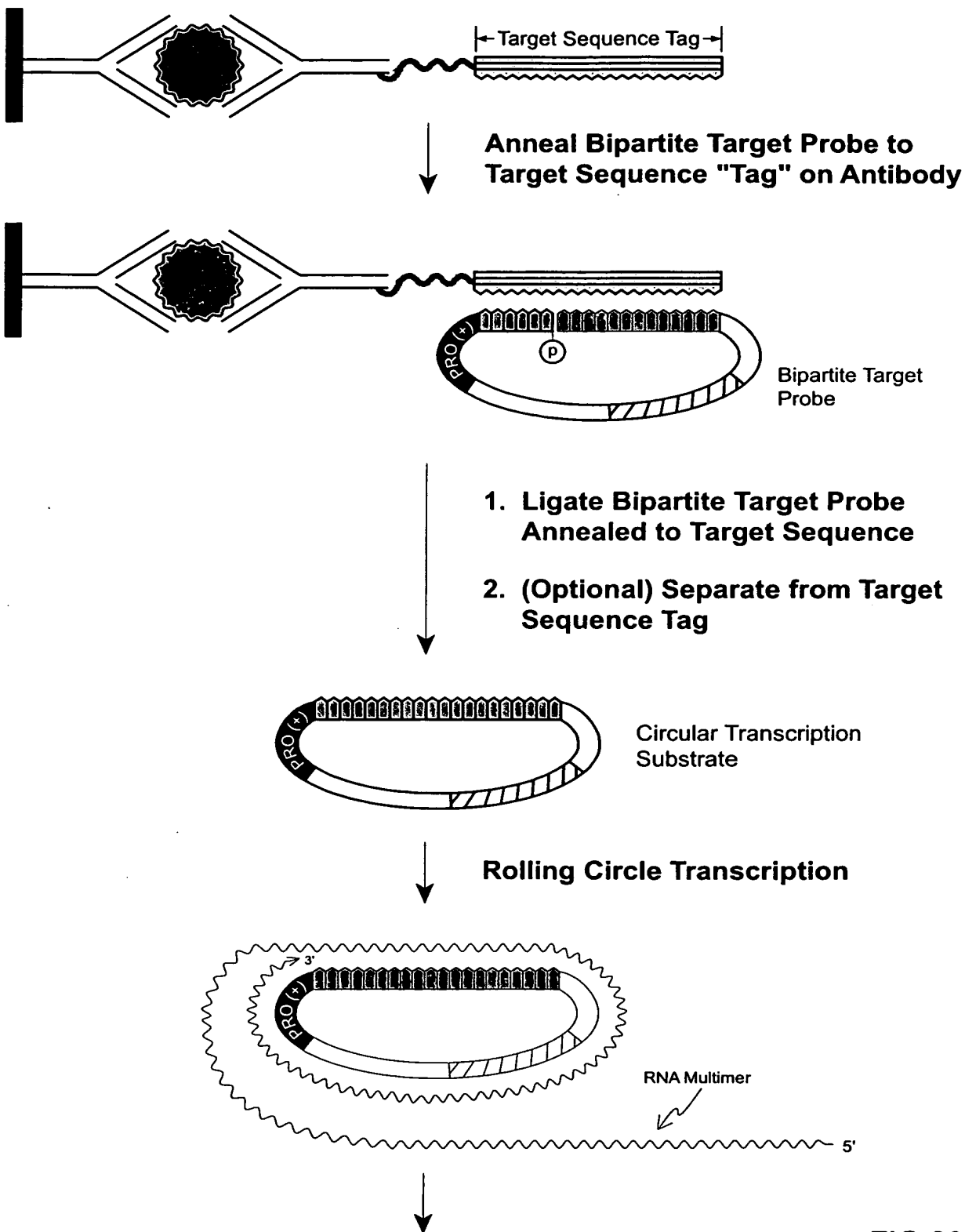


FIG 28